**Supplemental Material**

## Study Sample

Examinations for this study took place at follow-up between August 2001 and March 2003. A random sample of SAPALDIA2 participants aged 50 and over were offered 24-hour ECG recording. Amongst the 1837 persons (887 men, 950 women) who underwent such measurement, 1385 (75%) reported that they had never smoked cigarettes, pipes, cigarillos or cigars in the last 5 years and were included in the current study. Self reports were confirmed by end-expiratory carbon monoxide (CO) measurements for which EC50 Micro-Smokerlyzer (BEDFONT, Rochester, UK) were used. 91 participants were excluded because they had an end-expiratory CO of 7 ppm or higher, indicating that they may be smokers. Additional exclusions encompassed 62 subjects with insufficient ECG quality, 4 subjects with digitalis intake in the previous 30 days, 7 participants lacking SHS information, 2 subjects lacking BMI information, and 85 subjects without genotype due to lack of DNA. One participant was excluded because of a myocardial infarction in the previous 3 months. None of the participants had a cardiac pacer or anesthesia (narcosis or spinal anesthesia) in the eight days prior to ambulatory ECG recording. The final sample size for the study was n=1133 non-smoking subjects.

## Heart Rate Variability

For 24-hour ECG (Holter) recording, digital devices (Aria, Del Mar Medical Systems, Irvine, California) with a frequency response of 0.05 – 40 Hz and a resolution of 128 samples/sec. were used. The recorders were hooked up after the interview. Three leads ($V_1$, altered $V_3$ with the electrode on the left linea medioclavicularis on the lowest rib and altered $V_5$, with the electrode on the left linea axillaris anterior on the lowest rib) were recorded during 24-hours. Recordings with insufficient quality (n=32) or of less than 18 hours were not used (n= 30). Mean duration of the remaining recordings was 22.3 $\pm$ 2.1 hours. Participants were asked to follow their regular daily life and to fill in a time-activity diary during recording time.

All recordings were scanned through a StrataScan 563 (Del Mar) and interpreted using the interactive method, with a final visual check of the full disclosure. The length of each RR interval was manually validated during this step. Resampling was made at 4 HZ. Spectral analysis was performed by the Fast Fourier Transform method using sliding 256 PTAs windows for day and night periods. For 24 hours periods, calculations of RR intervals were made without sliding window, to allow measurement of ULF and of VLF. Only normal-to-normal intervals were used, with intervals excluded due to ectopy or artefacts being replaced by holding the previous coupling interval level throughout the time interval to the next valid coupling interval. The standard deviation of all normal-to-normal R-R (NN) intervals (SDNN), SDANN and rMSSD as well as the following frequency domain variables have been calculated: total power (TP; $\leq$ 0.40 Hz), ultra low frequency power (ULF;$\leq$ 0.0033 Hz), very low frequency power (VLF; 0.0033-0.04 Hz), low frequency power (LF; 0.04-0.15 Hz), high frequency power (HF; 0.15-0.40 Hz), and the ratio between LF and HF (LF/HF). Frequency

domain measures were given preference over time domain measures because of their easier interpretability. In order to avoid bias from the effects of methacholine administered in the preceding broncho-challenge test, we excluded the first two hours of recording.

**Blood markers and Genotype**

Non-fasting blood samples were collected from all consenting participants at follow-up and stored at -80°C. The following known cardiovascular risk factors were measured in serum as previously described (Felber Dietrich et al. 2006) : glucose, creatinine, uric acid, total cholesterol, triglycerides, high density lipoprotein cholesterol (if triglycerides ≤ 9.4 mmol/l), and high sensitive C-reactive protein.

DNA was extracted from EDTA-buffered blood using the PUREGENE $^{TM}$ DNA purification kit (GENTRA Systems, Minneapolis, USA). In all subjects *GSTM1* (UniGene Hs. 301961; UniGene 2008a) and *GSTT1* (UniGene Hs. 268573; UniGene 2008b gene deletions and a single nucleotide polymorphism in *GSTP1* (UniGene Hs. 523836; UniGene 2008c) leading to the amino acid substitution Ile105Val were genotyped on the ABI Prism 7000 sequence detection system using 5'nuclease real time PCR (TaqMan) assay (Applied Biosystems, Rotkreuz, Switzerland). Following primers and probes were used for *GSTM1*: forward 5'-GGACATTTTGGAGAACCAGACC-3' and reverse 5'-CTGGATTGTAGCAGATCATGCC-3' primers and *GSTM1*-specific probe 5'-VIC-TGGACAACCATATGCAG-TAMRA-3'; for *GSTT1*: forward 5'-GTCATTCTGAAGGCCAAGGACTT-3' and reverse 5'-GGCATCAGCTTCTGCTTTATGGT-3' primers and *GSTT1*-specific probe 5'-FAM-CACCTGCAGACCCC-TAMRA-3'; for *GSTP1*

Ile105Val: forward 5'-CCTGGTGGACATGGTGAATGAC-3' and reverse 5'-CAGATGCTCACATAGTTGGTGTAGA-3' primers and Ile105 -specific probe 5'-VIC-CTGCAAATACATCTCC-TAMRA-3'and Val 105 -specific probe 5'-FAM- CTGCAAATACGTCTCC-TAMRA-3'. *GSTM1*/*GSTT1* assays were repeated for all DNA samples carrying double homozygous *GSTM1* and *GSTT1* deletions using internal positive *GSTP1* controls. All double homozygous deletion carriers could be confirmed. In addition a 5% random sample of all DNA samples was regenotyped with highest reproducibility (>99.5%). Hardy-Weinberg equilibrium was tested using Arlequin (Version 2.000) software (Genetics & Biometry Laboratory, University of Geneva, Geneva, Switzerland). The distribution of *GSTP1* (Ile105Val) genotypes was found to be in Hardy Weinberg equilibrium.

**Statistical Analysis**

No sex-specific results are presented in the absence of sex-based differences in the reported effects. Sensitivity analysis were conducted including additional potential confounders (hypertension, former smoking status, blood markers (uric acid, high sensitive c-reactive protein, glucose, non-HDL cholesterol), alcohol intake, physical activity (enough to produce shortness of breath and enough to produce sweat), and medication (calcium channel blockers, ACE inhibitors, anti-arrhythmics, diuretics, sympathomimetics)).

Due to the skewed distribution of HRV values analyses were conducted based on log-transformed HRV values. Results are presented as percent change in HRV parameters compared to the respective reference groups by taking the antilog of the observed regression coefficients and expressing them as the difference to the baseline value of 100%.

Table 1: Percent difference[a] in time domain heart rate variability parameters according to *GST* genotypes, passive smoking and obesity, the SAPALDIA Cohort Study

| Genotype/ | SDNN | | SADNN | | r-MSSD | |
|---|---|---|---|---|---|---|
| Exposure | Δ% | 95%CI | Δ% | 95%CI | Δ% | 95%CI |
| *GSTM1* | | | | | | |
| deletion vs. no deletion[b] | - 1.4 | (-4.4, 1.7) | - 1.1 | (-4.3, 2.2) | 0.5 | (-4.6, 5.3) |
| *GSTT1* | | | | | | |
| deletion vs. no deletion[b] | - 2.8 | (-6.6,1.2) | - 2.1 | (-6.4,2.2) | -3.3 | (-9.0,2.8) |
| *GSTP1 Ile105Val* | | | | | | |
| Ile/Ile,Val vs. Val/Val | - 2.7 | (-7.8,2.7) | - 1.9 | (-7.4,4.0) | -1.4 | (-9.2,7.0) |
| *Second hand smoke exposure* | | | | | | |
| ≤2hrs/day vs. none | - 1.1 | (-6.3, 4.5) | - 0.6 | (-6.3,5.4) | - 6.2 | (-13.7,1.9) |
| >2hrs/day vs. none | - 4.5 | (-10.2,1.5) | - 3.4 | (-9.5,3.2) | 0.2 | (-8.7,9.9) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Obesity* | | | | | | |
| ≥ 30 vs. < 30 kg/m$^2$ | - 8.2 | (-11.8, -4.5) | - 8.2 | (-12.0, -4.2) | 0.6 | (-5.2,6.8) |

[a]adjusted for study area, gender, age and (age)$^2$, fruit intake, diabetes, beta blocker intake, and mutually adjusted for each other

[b] homozygous gene deletion

Table 2: Percent difference[a] in time domain heart rate variability parameters according to combination of *GST* genotypes with passive smoking and obesity, the SAPALDIA Cohort Study

| Genotype | Exposure | n | SDNN | | SDANN | | r-MSSD | |
|---|---|---|---|---|---|---|---|---|
| | | | Δ% | 95%CI | Δ% | 95%CI | Δ% | 95%CI |
| *GSTM1* | | | | | | | | |
| no deletion | no/low SHS | 504 | Ref. | - | Ref. | - | Ref. | - |
| no deletion | high SHS | 35 | - 1.8 | (-10.2,7.3) | 0.4 | (-8.8,10.4) | - 0.3 | (-13.9,14.3) |
| deletion[b] | no/low SHS | 551 | - 1.1 | (-4.2,2.0) | - 0.7 | (-4.0,2.7) | 0.4 | (-4.3, 5.3) |
| deletion[b] | high SHS | 35 | - 7.3 | (-14.6, 0.5) | -6.7 | (-14.5,1.9) | 2.6 | (-9.4,16.2) |
| p trend[c] | | | 0.09 | | 0.19 | | 0.71 | |
| no deletion | not obese | 437 | Ref. | - | Ref. | - | Ref. | - |
| no deletion | obese | 102 | - 3.2 | (-8.6,2.5) | - 2.3 | (-8.1,3.9) | - 5.9 | (-2.9,15.5) |
| deletion[g] | not obese | 473 | + 0.6 | (-2.8,4.1) | + 1.2 | (-2.4,5.0) | + 2.4 | (-2.7,7.9) |

| | | | | | |
|---|---|---|---|---|---|
| deletion[b] | obese | 121 | -11.7 (-16.3, -6.9) | - 11.8 (-16.6,-6.6) | - 1.3 (-9.9,7.0) |
| p trend[c] | | | 0.25 | 0.39 | 0.95 |
| | | | | | |
| *GSTT1* | | | | | |
| no deletion | no/low SHS | 871 | Ref. - | Ref. - | Ref. - |
| no deletion | high SHS | 63 | - 4.9 (-11.0,1.7) | - 4.0 (-10.7,3.1) | - 0.9 (-10.5,9.7) |
| deletion[b] | no/low SHS | 184 | - 2.9 (-6.8,1.3) | - 2.3 (-6.5,2.2) | - 4.0 (-9.9,2.3) |
| deletion[b] | high SHS | 15 | - 4.5 (-16.3, 9.1) | -1.7 (-14.8, 13.3) | 5.8 (-13.7,29.6) |
| P trend[c] | | | 0.08 | 0.24 | 0.60 |
| | | | | | |
| no deletion | not obese | 748 | Ref. - | Ref. - | Ref. - |
| no deletion | obese | 186 | - 6.4 (-10.3, -2.2) | - 6.1 (-10.3,1.7) | 0.03 (-6.3,6.7) |
| deletion[b] | not obese | 162 | - 0.5 (-4.8,4.1) | 0.4 (-4.3,5.4) | - 4.0 (-10.2,2.8) |
| deletion[b] | obese | 37 | -17.6 (-24.5,-10.2) | - 17.5 (-24.9,-9.5) | - 0.5 (-12.8, 13.6) |
| p trend[c] | | | 0.15 | 0.34 | 0.56 |

| | | | | | |
|---|---|---|---|---|---|
| *GSTP1* | *Ile105Val* | | | | |
| Val/Val | no/low SHS | 87 | Ref. - | Ref. - | Ref. - |
| Val/Val | high SHS | 11 | -10.2 (-23.7, 5.6) | - 9.3 (-23.9,8.0) | 5.4 (-17.8,35.2) |
| Ile/Ile,Val | no/low SHS | 969 | - 3.6 (-8.9,2.0) | - 2.8 (-8.5, 3.3) | - 0.7 (-8.9,8.4) |
| Ile/Ile,Val | highSHS | 67 | - 7.0 (-14.4, 1.0) | - 5.0 (-13.1, 3.8) | - 0.4 (-12.2, 13.2) |
| P trend[c] | | | 0.10 | 0.29 | 0.98 |
| | | | | | |
| no deletion | not obese | 79 | Ref. - | Ref. - | Ref. - |
| no deletion | obese | 19 | - 1.7 (-10.8, 16.0) | 2.9 (-10.6,18.6) | 9.5 (-10.3,33.7) |
| deletion[‡] | not obese | 831 | - 0.6 (-6.4,5.6) | 0.5 (-5.8,7.3) | 0.3 (-8.5,9.9) |
| deletion | obese | 204 | - 9.6 (-15.6,-3.2) | - 8.7 (-15.1,-1.7) | 0.2 (-9.7,11.1) |
| p trend[c] | | | 0.68 | 0.96 | 0.93 |
| | | | | | |
| Obesity | | | | | |
| No/low SHS | not obese | 855 | Ref | Ref | Ref |
| No/low SHS | obese | 200 | -4.3 (-10.9,2.8) | - 3.9 (-10.9,3.7) | 4.1 (-6.6,16.1) |

| | | | | | |
|---|---|---|---|---|---|
| High SHS | not obese | 55 | 1.8 (-4.0,8.0) | 2.4 (-4.0,9.1) | 2.3 (-6.7, 12.1) |
| High SHS | obese | 23 | -2.9 (13.5, 8.9) | 0.4 (-11.3,13.6) | - 4.4 (-19.8,14.0) |
| p trend[c] | | | 0.48 | 0.87 | 0.98 |

[a] adjusted for study area, gender, age and $(age)^2$, fruit intake, diabetes, beta blocker intake, and mutually adjusted for each other GST polymorphisms not mutually adjusted for each other. *GST*/SHS models adjusted for BMI as a continuous variable. GST/obesity models adjusted for SHS.

[b] homozygous deletion

[c] the p values for trend are derived from entering a cross-categorized variable coded as 1, 2 and 4, respectively for subjects exhibiting 0, 1 or 2 at-risk characteristics.

**References Supplemental Material**

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